SYNOPSIS

"Studies on Phosphate Solubilizing Halophilic bacteria as improved Bioconditioner for Agricultural soil."

A Synopsis

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Abstract

Due to use of heavy chemical fertilizer soil becomes alkaline. Phosphorous is one of the major nutrient for the plant growth and it plays an important role in plant metabolism. The use of phosphate solubilizing organisms to increase the availabilityof phosphorous to crop has been fairly wide practice. Now a days the heavy use of chemical fertilizers cause the alkaline soil it is necessary to study the phosphate solubilization in alkaline condition. In alkaline condition enzyme alkaline soil it is necessary to study the phosphate solubilization in alkaline condition. In alkaline condition enzyme alkaline phosphatase produced by micro organism. The natural resources of sulphur are fast depleting & it is required for many other industries including pharmaceuticals. A low recovery of applied phosphatic fertilizer also encourage the use of phosphomicro organism.

Phosphate solubilizers are economical ecofriendly & have greater agronomic utility to compensate the expensive inorganic sources of P fertilizers.

Alkaline phosphatase can be found on the outer surface of the cell where they cleave a variety of monoesters in surrounding outer. Alkaline phosphatases (E.C.3.1.31) are periplasmic enzymes that are found in organisms ranging from bacteria (Including extremophile) to mammals. Alkaline phosphatase (AP) is a key-enzyme in the marine environment which catalyses the liberation of orthophosphate from organic phosphorous compounds.

Halophilic bacteria produce a wide range of extra cellular salt-tolerant hydrolytic enzymes such as amylases, proteases, nucleases, phosphatases, lipases, dalases, pullulanasesxylanes, that have diverse potential vsages in different areas such as food technology, feed additive and chemical industries.

Key words : phosphate solubilizing bacteria, alkaline phosphatses, Halophilic bacteria.

1. Introduction:

Due to use of heavy chemical fertilizer soil becomes alkaline, phosphorous is one of the major nutrient for the plant growth and it plays an important role in plant metabolism. The situation is more complicated because the natural reserves of raw materials used for phosphatic fertilizer are limited. Soils containing both organic and inorganic forms are compounds of phytins, phospholipids and nucleic acid which come mainly from decaying vegetation. Plants take only solubleform of phosphorous. Phosphate solubilizing micro-organisms have ability to convert the insoluble form of phosphorus to soluble form of phosphorous and make it available to plant.

The use of phosphate solubilizing organisms to increase the availability of phosphorus to crop has been fairly wide practice. The phosphate solubilizing organisms solubilize the phosphate at high rate in the optimum condition of temp, and pH. The phosphate solubilizationby these organisms is brought about by production of organic acids. Some of which act as powerful chelator of calcium and thus make increased quantity of phosphorus available to plants. The extra average grain yield may be obtained due to use of phosphomicro organism. Rock phosphate is one of the raw material for the production of phosphatic. More over high cost involved in the transportation of rock phosphate. The phosphate solubilizing organism may produce growth promoting hormones such as auxins and gibberlins.

Therefore the beneficial effect of there organism may also be due to the production of growth promoting substance beside phosphate solubilization.Now a days the heavy use of chemical fertilizers cause the alkaline soil hence it is necessary to study the phosphate solubilization in alkaline condition. In alkaline condition enzyme alkaline phosphatase produced by micro-organism.A great variety of phosphates produced by micro-organism. The acid and alkaline phosphatases of micro-organism are so called because of optimum activity at an acid and alkaline pH respectively. Alkaline phosphatase is the enzyme which hydrolyzes a number of phosphate ester to yield orthophosphate and an alcohol.

Orthophosphate also phosphorylates the enzyme at particular serine oH group of the active center and both ester phosphate and orthophosphate evidently

bind at the same enzyme site from similar complexes, after binding the substrate by reaction (1) enzyme phosphorylated and the alcohol group is freedin (2) then phosphoryl enzyme is hydrolyzed by reverse reaction (2) giving orthophosphate which is still bound to enzyme. Decomposition of his complex yields free enzyme and orthophosphate.

The optimization of phosphate solubilizing micro-organism can be done with the different parameter. Effect of different temperature, pH and heavy metal ions on the phosphate solubilization can be check. The optimum temperature for the phosphate solubilization activity is 27^0c.Negligible activity as found at lower temperature. The lower and higher temperature caused delay in the expression of phosphate solubilizing activity. The 37^0 C was found to be most stable temperature for phosphate solubilization. The temperature effect varies with different temperature.

The effect of different metal ions on the phosphate solubilization aredifferent. The Zn treatment significantly enhances the phosphate solubilization activity by 9.9 to 57%. The phosphate solubilization under the treatment in presence of phosphobacteriainereased up to 50%. The availability and absorption of phosphorous by plants is adversely affected by the presence of iron and aluminum at low pH and calcium at high pH. That is in acidic condition iron and aluminum causes adverse effect on phosphorous uptake by the plants.

Another soil property affecting the phosphorous availability is pH. The pH form maximum soil phosphorous is 6.5 to 7.0 but now days increased use of chemical fertilizer cause alkaline soil. Hence phosphorus available for the plants is limited so it was essential to study on the phosphate solubilization in alkaline condition so bacteria solubilize the phosphate in alkaline condition.

The need for utilization of micro-organisms in solubilization of insoluble phosphates has arisen because of that to basic ingredients sulphur and rock phosphates needed for manufacture of phosphatic fertilizer are limited. The nature resources of sulphur are fast depleting and it is required for many other industries including pharmaceuticals. Low recoveries of applied phosphatic fertilizers also encourage the use of phosphomicro-organism.

Therefore the attempt has made to study or optimization of phosphate solubilization by bacteria have objectives of project are biochemical test organism determination of protein assay of acid and alkaline phosphatase and effect of different pH temperature and metal ions on phosphate solubilizaiton.

2. Review of literature and Development in the subject (Previous work done in the relevant area):

Phosphorous (P) is the one of the most important macro nutrients required by plants. Though soils generally contain high amount of P (0.005%) only small amount (0.1%) of the total P is available for plant uptake (Changand yang 2009) thus it is considered to be the most limiting nutrient. A large portion of applied inorganic soluble phosphate fertilizer to soil is rapidly becoming unavailable to plants, which leads to excess application. Recently emphasis has been paid to utilize unavailable P forms thorough the action of phosphate solubilizing micro-organisms (PSMS).

PSMS are naturally found in the soils, however, their growth and solubilization highly vary with environmental conditions.Such as temp, pH and salt concentration of the soils. Though, there are some information regarding the stress tolerant to PSMS such Rhodotorulasp PS4 is highly tolerant to temperature, pH and salt variations (Mundra et al 2011).

Phosphate solubilizers are economical, ecofriendly and have greater agronomic utility compensate the expensive inorganic sources of P fertilizers. Phosphate solubilizing micro-organisms (PSM) have attracted attention in semi arid regions and endowed to enhance the crop yields (Fasim et al. 2002 ; khan et.al 2006). Phosphate solubilizing micrp-organisms have estabilised (Iguala et.al; 2001) phosphate solubilizing bacteria (PSB) are the group of PGPR in rhizosphere. Secreation of organic acid and phosphatases to solubilize insoluble phosphate to soluble forms are common in this group. (Kim et.al. 1998).Although sever phosphate solubilizing bacteria occur in soils their numbers are not adequate to compete with bacteria commonly established in the rhizosphere (Glick et.al ; 1995).

The finding to hyperthermophilicarchaea living at extreme high temperature has revolutionized scientific thought in this area, and leads many scientists to study its unique survival systems. Due to thermal properties, and physic-chemical stability in particular area enzymes from extremophiles offer great potential for basic research as well as for industrial application. Recently we have identified and characterized a unique archaealdissacharide catabolic pathway from pyrococussp ST 4 in whichTwoenzyme, kojibiose, phosphorylase and phosphoglucomutase, regulate the kojibiosein the gycolyticpathway(Jung et.al 2014). This was the first report on an archaneal disaccharide catabolic system that relied on phosphorylation.

Phosphorous limitation is phytoplankton has been studied using a variety of methods including nutrient incubation experiments and direct measurements of phosphorous uptake cellular phosphorous content, nutrient ratios, and /or the presence of alkaline phosphatase activity (APA) (Vincent 1981)of these, alkaline phosphatase (AP) is the only biochemical indicator of phosphorous limitation in phytoplankton, in the laboratory (Kuenzlev, 1965) as well as in the field (Berman 1979), petterson, 1980, Vincent, 1981). Further most alkaline phosphatases appear to be inducible, in contrast to acid constitutive phosphatases (Cembella et.al. 1984).

Alkaline phosphatases can be found on the outer surface of the cell where they cleave a variety of monoesters in surrounding water (kuenzlev, 1965), thereby allowing phytoplankton to utilize organic phosphates when inorganic phosphate is no longer available (Kuenzter and perras, 1965)

Halophilic bacteria live and grow in high salt environments, and are classified into essentially three groups (slight halophiles, moderate halphiles and extreme, halophiles, Mishra & champagne, 2009). These halophiles exhibit different optimal salt concentration for growth as fallows 2-5% salt for slight halophiles (seawater contains – 3.5% salt), 5-20% salt for moderate halophiles and >20% up to saturated salt solutions of 36-39% depending to temperature. For extreme halophiles, (Hayashi et.al 1973, Vreeland K. Hochstein, 1992, Ollivier et.al 1994, Seckbach, 2011). To adapt to an environment containing

almost saturated salt proteins from extreme halophiles and from the extracellular and periplasmic fractions of moderate halophiles exhibit abundant acidic surface amino acids (e.g. Glua ASP) that promote solubility in high salt environment (Lanyi, 1974).

Halophile proteins typically also exhibit a reduced core hydrophobicity, as salt stabilizes such interaction (Paul et.al, 2008) thus an abundance of negative surface charge and reduced core hydrophobicity are two of the characteristic features of halophile proteins in comparison to non-halophiles and other extremeophiles.

Alkaline phosphatases (E.C. 3.1.3.1) are periplasmic enzymes that are found inorganisms ranging from bacteria (including extremophile) to mammals. As of 2013, 66 crystal structure of AP have been deposited in the protein Data Bank (PDB).

Among these structures APS from the extreme halophile *HalobacteriumSalinarum* (HS AP PDB entry) 2 x 98; wende et.al. 2010.

Moreover halophilic proteins generally require a high salt concentration to fold and enable enzymatic activity, we recently showed that AP from the moderate halophile, *Halomonas PP* 593 (HaAP) maintains enzymatic activity over a widerange of salt concentrations (1-4 M Nacl; Ishibashi et.al; 2005, 2011).

In phosphorus – depleted oceans with hgihrates of nitrogen fixation (cotner-et al. 1997; karl et.al 2001, vidal et.al; 2003 :Ammevman et.al 2003). And in coastal areas with high nitrogen availability (Hernandez et.al 1996 Ammerman and Glover, 2000; Cotner et.al 2000;Glibert el.al 2004, Labry et.al.; 2005, Boyer et.al., 2006).

Phytoplankton and bacteria can satisfy their phosphorous (P) requirements by producing hydrolytic enzymes such as alkaline phosphatase (AP) to sequester organic P. Phosphatases have broad range of organic substrate specificities; thus hydrolyze a wide variety of organic. Phosphomonoester or PMES (Ammerman, 1991:Shan, et.al 1994).

Phosphatase enzymes are attached to cell surface or are freely dissolved in the water column resulting from cell lysis excretion (Jansson et.al. 1988; Li

et.al; 1988; Hoppe, 2003). Upon enzyme hydrolysis, PMES release inorganic phosphate in to water, along with their by increasing inorganic availability for Planktonic as well as benthic organisms in shallow marine systems. Organic p constitutes the major component of total P in the sediment and water column of many lakes when soluble inorganic P concentrations within the water column are low, plankton may produce phosphatase ensymes, which hydrolyze, organic P compounds with the release of inorganic P (kuenzler and perras, 1965, Reichardt, 1971). Thus the measurement of alkaline phosphatase activity (APA) has been used as a tool to indicate P limitation and potential organic P mineralization through enzymatic hydrolysis (Gage and Gorham 1985, Healey and Hendzel, 1979a).

Enzymatic hydrolysis of organic P compounds is usually rapid under oxygenated water column conditions, and rate of hydrolysis can potentially decrease with oxygen depletion (Pulford and Tabatabai, 1988). This may be particularly important at the sediment water interface where (DO) concentration are often depleted.

Alkaline phosphatase (AP) is a key-enzyme in the marine environment which catalyzes the liberation of orthophosphate from organic phosphorous compounds. The total phosphatase activity in sea water results from a mixture of phosphatases localized on the cell surfaces of bacteria and from dissolved enzyme (Hoppe, 2003). Phosphatases are Inducible catabolicEcto enzymes of aquatic micro-organisms (Boavida, 1990, Vazaues 2000). The distribution of phosphatase activity among the particulate and the dissolved factions is highly variable. Dissolved fraction can contribute up to 70% of the total (APA) to release bioavailable phosphorous (Siuda 1984).

Halophilicarchaea inhabit the most saline environment on earth, including solar salterns and natural sat lakes. Like many other habitats where archaea are found, salt lakes were once thought devoid of life, In 1936, Benjamin Elazarivolcani published the first report of microbial life in the dead sea (Elazari-volcani 1936). His work was commemorated by the haming of Haloferaxvolvani which was isolated from Deadsea mud in 1977

(Mullakhanbhai& Larsen, 1975). In fact, the discovery of halophilicarchaea predates the proposal of the domain Archaea by carlwoese in the late 1970S (woese& fox 1977, woese et.al 1990).

For instance *H. salinarum* was unwittingly discovered in 1922 as a red discoloration of salted fish (Harrison & Kennedy, 1922). All halophilicarchaea are members of the Euryarchaeota and somewhat confusingly, the family halobacteria (Orcn et.al 2009). In this review, we will use instead the term haloarchaea unless when referring to the genus Halobacterium.

Archaea are not alone in hypersaline environments, theyshare this habitat with bacteria, fungi and algae. In contrast to most halophilic bacteria and eukaryotes, haloarchaea maintain an osmotic balance with their medium by accumulating equimolar salt concentration in the cytoplasm (chivstian&waltho, 1962; oren 2008). The salt out strategy is energetically costly and less suitable at saturating salt concentrations which is probably why halo archaea predominate under hypersalineconditionsoren 1999). There are notable exceptions, the bacterium *salinibacterruber* uses the archael salt in strategy and coexists with haloarchaea at near saturating salt concentrations. (oren et.al 2002).

A significant motivation for working on haloarchaea is the sophisfication of their genetic systems. They are easy to cultivate in the laboratory have fast growth kineties (Robinson et.al 2005), and are resistant to contamination by nonhalophilic micro-organisms (of course, cross-contamination of haloarchaeal strain with in the laboratory remains a constant treat). Halophiles were the first archaal group in which routine transformation with foregin DNA was possible (charlebois et.al 1987; cline et.al 1989).

3. Objectives of Research / Proposed Hypothesis:

Seems to be essential as the heavy use of chemical fertilizers the cause of the alkaline soil.In view of this various agrimicrobial technological issues need to be reoriented to increase its efficiency primarily the phosphate solubilization

in alkaline condition posses problem as pH level for maximum available soil phosphorus at 6.5 to 7.0 resulted inlimited phosphorus availability.

Therefore it necessitate the study of the phosphate solubilization in alkaline pH range particularly by PSB in alkaline condition using enzyme alkaline phosphatase which hydrolyse number of phosphate ester to yield ortho phosphate and an alcohol.

Secondly the world wide spread of inflation initiated several fold increase in petroleum product prices thereby depicting its striking influence on the prices of chemical fertilizers which have nearby double during the 5 to 10 years. Which demands to search for cheaper alternative resources of nitrogen to meet the need of crops.

This has rejuvenated soil microbiology to tap amount the biological fixation nitrogen again.

Now a days, soil texture has been change due to heavy use of chemical fertilizers in many states of our country. Such regions shows increase in soil pH up to 9.0 to 10.0, hence become alkaline & saline also. Hence use of bio fertilizers alone or in combination with soil conditioner is suddenly provoketed. But most of the marketed standard bio-fertilizers represent azotrophic species. Which could show required optimally at pH ranges from 6.8 to 7.4 in laboratory conditions and field trails.

Significance and efficiency of use or role such standard marketed bio-fertilizer will be in question in such alkaline soil which represents pH range between 9.0 to 10.0. Therefore use alkaline PSB in combination as soil conditioner could be a solution to rejuvenate the science of bio-fertilizer technology. Such trained biological agents could act in many ways by fascinating reclamation of saline soil, soil fertility and bio-control agent as well.

Today there is urgent need for the development such a possible alkaline phosphate solubilization bacteria as soil conditioner on large scale for the reason that it could facilitates following significant demand as agriculture in the country in present scenario.

Presence of such bio-fertilizer promotes seed germination and plant growth in alkaline pH.

Developed strain could be potential candidate to develop soil conditioner for crops due to its significant potential as nitrogen fixer and phosphate solubilizer along with growth promoting substances, alkaline PSB studied are also tolerant to high salts. Hence, it thrives even in alkaline soil.

The potential roles of phosphate solubilizing bacteria as phosphate solubilizers in enhancing crop production so following are the objectives of research.

1. Enrichment, screening and isolation of Halophilic phosphate solubilizing bacteria from hyper saline habitat.
2. Identification of selected Halophilic phosphate solubilizing bacteria by using biochemical and molecular markers.
3. Physico-chemical characterization and standardization of phosphate solubilizing activity of isolate.
4. Optimization of phosphatase activity by using statistical programmes (factorial design)
5. Scale up and production of Halophilic phosphate bacteria that is phosphate solubilizing bacteria by using shake flask and bioreactor.
6. Investigation for testing suitability of isolate as potential candidate as microphosbioinoculant.
7. Pot and field trial of Halophilic phosphate solubilizing bioinoculant as alkaline soil conditioner.

4. Methodology to be adopted:

i. Isolation and screening of phosphate solubilizing bacteria.

The hyper saline soil samples or water more collected and transferred under sterile conditions or laboratory zero point one mililitor of the soil sample was spread on modified pikovskaya gar plates for phosphate solubilization assay described by nautiyal (5). Bacterial colonies forming

halo zones around the bacterial growth here considered to be phosphate solubilizes.

ii. Measurement of culture growth and phosphatase activity.

Selected efficient bacterial strain was inculated in the pikovskayas agar medium & incubated at 37^0 C, growth and enzyme activity were assessed for 24 hours.

iii. Effect of various pH on phosphatase activity.

The phosphate solubilizing positive colonies were incubated in pikovskayas broth and maintained in different pH (2-10) and incubated at 37^0c for 24 hours& kept in shaker. After incubation the culture was centrifuged for the crude enzyme. The crude enzyme activity was analyzed as mentioned by Black et.al (1999).

- Effect of temperature on phosphatase activity.
- Effect of various nitrogen sources on phosphates activity.
- Effect of various concentration of tricalcium phosphate on phosphatase activity.
- Effect of different concentration of superphosphates on phosphatase activity.
- Phosphate solubilization under stressconditions. Phosphorous solubilization was determined using phosphomolydatebluecolourmethod (Murphy and Riyley 962). Samples from culture grown in NBRIP medium were diluted 1:1 (v/v) using NHCL to dissolve the residual in soluble phosphate and absorbance wasrecorded at 660 nm against a blank to measure the growth to strain (Rodrigyez et.al 2000).

iv. Biochemical characterization:

Gram straining was performed to study the morphological characteristics of the selected isolates various biochemical test were performed citrate utilization, production of catalase, oxidase, indole, Urease, acetionor acid production and gelatinliquiefaction. Various carbon sucrose were tested, source, glucose, arabinose, Maltose, lactose (CappuccinoJ.C.,2006)

- Salt tolerance
- pH range
- proteolytic assay
- Lipolytic assay
- Starch hydrolysis
- Phosphate solubtization
- Siderophone analysis
- Heavy metal tolerance
- 165 rRNA gene sequencing
- Analyses of nutrients and bulk alkaline phosphatase activity (APA) alkaline phosphatase activity was measured according to the method of petterson (petter son 1980) methyl umbelifery phosphate MUPC10^{-4}mol 1^{-1} J in 0.1 mol 1^{-1}tris buffer pH 8.3 was used.
- Aubundance of phosphate solubilizing bacteria (Gautam et.al 2003) Desouza et.al (2000).
- Alkaline phosphatase activity (Taga and kobori 1976).
- Optimization of growth conditions.
- Kineties of phosphate solubilization.
- Effect of calcium salts on phosphate solubilization.
- For phosphate solubilization, phosphobacteria produce phosphatase enzyme.(Tabatabai and Brimer 1969)

5. Importance of Study / Society application:

During the last 50 years considerable efforts have been made to increase agricultural production. The awareness and education of the farmers has resulted in the increase in demand for chemical fertilizers. In India from 1950 to 1995 the demand for chemical fertilizers has increased approximately 25 times which has resulted four times increase in crop productivity. Enhanced consumption of chemical fertilizers has created certain problems. Production of chemical

consumes nonrenewable energy source like petroleum. Most of the chemical fertilizers are to be imported on the lost of foreign currency. Large amount of chemical fertilizers applied in the soil is immobilized & not available to the plant. Moreover there is an increasing demand for chemical fertilizers which cannot be fulfilled in the country. The cost is unbearable to the poor farmers of India and the excessive use of chemical fertilizers creates a number of pollution problems.

Microbial fertilizers have a number of advantages over chemical fertilizers. Microbial fertilizers are very cheap, production method is very simple and it creates no pollution problems. They supply other nutrients may control platnpathugens. Also supply vitamins and plant growth harmones and prevent soil erosion by producing capsular polysaccharides. They also convert immobilized chemical fertilizers in to soluble forms and make them available to the plants.

Phosphate suppliers

The phosphorous is second important plant nutrient. There are two types of micro-organisms making available this plant nutrient.

Phosphate solubilizers soil contain large amount of insoluble inorganic phosphates. Also soil contain immobilized organic phosphorous. Phosphate solubilizing micro-organisms (P.S.M.) are capable of solubilizing tricalcium, aluminum, iron and rock phosphate and mineralizing organic phosphorous and making the phosphorous present in the soil available to the crops.

Phosphate solubilizing bacteria is a potent thermo, acid, alkali and salt tolerant phosphate solubilizing bacteria which retains its phosphate solubilizing capacity over a wide range of pH, temp., and salt concentrations.

Halophilic bacteria produce a wide range of extracellular salt- tolerant hydrolytic enzymes such as amylases, proteases, nucleases, phosphatases, lipases, Dnases, pullulanases, xylanases, that have diverse potential usages in different areas such as food technology, feed additives and chemical industries.

- Thus alkaline phosphatase plays a prominent role in the recycling of organic phosphate.
- Haloarchaemaintain an osmotic balance with their medium by accumulating equimolar salt concentration in the cytoplasm.

- Denaturation resistant properly as well as higher activity and stability in the presence of high salt concentrations make halophilic enzyme attractive for the industrial applications.
- Halophilic bacteria are a potential source of extracellular hydrolases like proteases with a wide arry of industrial applications.
- These micro-organisms can be used for a wide range of applications including protein and /or enzyme stabilizers, comestic actives, therapeutic agents, bioremediation and also in improving the salinity tolerance of crop plants by gene transfer.
- Due the effect of nutrients on plants vegetative& reproductive growth and changes in yield product, supplying the nutrients required by plants and soil and create a balance between them is important. So in order to achive this goal and supplying bio-fertilizers to balance between nutrients these fertilizers will be used as a replacement for chemical fertilizers in agriculture.
- Nearly 40% of world's surface has salinity salinization of soil is a serious problem & is increasing gradually in many parts of the worlds particularly in acid and semiarid areas. At present out of 1.5 billion hectares of cultivated land around the worlds, about 77 million hectares is affected excess salt content.

At high salinity level, it was found that treatments supplied by bio-fertilization with yeast decreased the adverse effect of salinity. Halophilic micro-organisms are already in use for some biotechnological processes, such as commercial production of carotene, polymers, enzyme, compatible solutes.

- halophiles are becoming increasingly valuable for bioremediatiation since many industrial processes release salts as contaminated brine effluent in to the environment. Another growing area for applications of halophiles is in the development of sustainable bioenergy technology to address concerns about petroleum shortage & global warming.
- Their high stability at extreme conditions such as high salt concentrations, low water availability and high temp, make halophile enzymes, including

cellulases, lipases, amylases & restriction enzymes, particular interest in Industrial & biotechnological applications.

6. Proposed work Plan / Formulation and Structure of Study:

First Year

1. Collection of review of literature of phosphate solubilizing Halophilic bacteria which solubilize phosphate.
2. Primary screening of hyper saline phosphate solubilizing bacteria.
3. Identification of selected Halophilic phosphate solubilizing bacteria by using biochemical and molecular markers.
4. Physico-chemical characterization and standardization of phosphate solubilizing activity of isolate.

Second Year

1. Optimization of phosphatase activity by using statistical programmes (factorial design)
2. Scale up and production of Halophilic phosphate bacteria that is phosphate solubilizing bacteria by using shake flask and bioreactor.
3. Investigation for testing suitability of isolate as potential candidate as microphosbioinoculant.
4. Pot and field trial of Halophilic phosphate solubilizing bioinoculant as alkaline soil conditioner.
5. Thesis writing and submission to University.

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